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Advances in Research of Biological Activity, Action Mechanism and Structure - Activity Relationship of Lentinan

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Abstract *Lentinula edodes* is the second largest edible mushroom in the world and is widely used as food and medicine. Modern research shows that lentinan (LNT) is the main active component of *L. edodes*. It has anti-cancer, treatment of diabetes, intestinal protection, anti-inflammatory, anti-oxidation, anti-aging, hepatoprotective, immune-regulating effects. In this review, the biological activity, action mechanism and structure-activity relationship of LNT in recent years are reviewed. On this basis, the existing problems were discussed, and the future research and application of LNT were prospected. Finally, it is hoped that this review will promote the in-depth study of LNT and provide a reference for its development as a drug and functional food.

Key words Lentinan, Biological activities, Action mechanism, Structure-activity relationship

1 Introduction

Shiitake mushroom [*Lentinula edodes* (Berk) Sing], also known as shiitake and shioshin, belongs to the genus *Shiitake* in the family of white mushrooms (portulaca) (Tricholomaceae)[1], and is the world second-largest cultivated edible fungus, accounting for about 25% of global production[2]. As early as in the *Compendium of Materia Medica*, it is recorded that shiitake mushroom has the effect of "sweet, flat, non-toxic, can benefit qi and not hunger, cure the wind and break the blood, dissolve phlegm, benefit the taste and help food, and manage the incontinence of urination". Shiitake mushroom, as a medicinal food[3], has high nutritional value and contains a variety of biologically active compounds, lentinan (LNT), dietary fiber, ergosterol, vitamins B1, B2, and C, folic acid, niacin, and minerals[4]. Beginning with the first extraction of shiitake crude polysaccharide from shiitake mushroom by hot water extraction method in 1969 and proving its activity in inhibiting sarcoma in mice[5], a large number of scientific experiments have been carried out since then with LNT as the target to study its extraction method, biological activity and structure. In this paper, we reviewed the activities of LNT from anticancer, treatment of diabetes, intestinal protection, anti-inflammatory, antioxidant, anti-aging, hepatoprotective, immunomodulatory and so on, with a view to provide a reference for the further development, utilization and in-depth research of LNT.

2 Biological activity of LNT

2.1 Anticancer activity Despite our growing understanding of cancer, the prevalence of cancer continues to increase each year[6]. Data from the World Health Organization (WHO) in 2020 indicated that there were nearly 19.3 million diagnosed cases of cancer and approximately 10 million cancer deaths worldwide, with the top five diagnosed cancers being: breast (11.7%), lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach (5.6%)[7]. Traditional cancer treatments include surgical resection, chemotherapy and radiotherapy. Among them, surgery and radiotherapy are more damaging to the human body, and chemotherapy also causes unavoidable damage to the body during treatment, such as hair loss and gastrointestinal toxicity[8]. In recent years, natural products have become a top priority in the development of anticancer drugs due to their well-defined anticancer effects and abundant candidate resources[9], so it is important to search for natural anticancer products that are safe, efficient, with few side effects and highly targeted. According to studies, LNT has certain biological activities against pancreatic cancer, thyroid cancer, human glioma, leukemia, colorectal adenocarcinoma, hepatocellular carcinoma, cervical carcinoma, prostate cancer, and breast cancer, and exerts its anticancer activities mainly through value-added inhibitory effects, cell cycle modulation, inhibition of cell migration, induction of apoptosis, and activation of immune cells. Therefore, LNT can be used as a natural antitumor agent in cancer symptom reduction drugs or and functional food industry. The action mechanism and effects of antitumor activity of LNT are shown in Table 1.

2.2 Therapeutic diabetic activity Diabetes mellitus (DM) is a chronic metabolic disease with abnormally elevated blood glucose levels, and more than 400 million people worldwide suffer from diabetes mellitus, and its morbidity and mortality rates have been on the rise year by year, while the most common cause of death is chronic complications, which are most fundamentally vascular[28-29]. Studies have shown that LNT attenuates high glucose-

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Table 1 Recent studies on anti-cancer effect and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
ASPC-1	*In vitro*	1, 10, 20, 40, 80, 160, 320 μg/mL	Enhancing the inhibitory effect on the proliferation of ASPC-1 cells and promoting apoptosis	[10]
Mouse	A mouse model of thyroid cancer was established by injecting thyroid cancer TT cell suspension into the back	20 mg/kg m_b	Regulating Wnt-β-catenin signaling pathway to inhibit tumor growth	[11]
SHG-44	*In vitro*	2, 4 mg/mL	By regulating the cell cycle of SHG-44, it can inhibit the proliferation of SHG-44 cells and inhibit the migration of tumor cells	[12]
HL-60	*In vitro*	15, 30, 45 mg/L	Inhibiting PI3K-Akt signaling pathway induces apoptosis of HL-60 cells	[13]
SW837	*In vitro*	0.4 – 4.0 mg/mL	Regulating the expression of immunogenic death-related molecules, immune checkpoint molecules and costimulatory molecules	[14]
Male mice	A tumor-bearing mouse model was established by subcutaneous inoculation of H22 cells	50, 100, 200 mg/kg m_b	Inhibiting Akt pathway promotes mitochondrial apoptosis pathway and inhibiting tubulin polymerization	[15]
Female mice	Tumor-bearing mouse model was established by intraperitoneal injection of H22 cells	5, 50, 500 μg/mL m_b	Up-regulation of Bax and down-regulation of Bcl-2 expression arrested tumor cells in G_2-M phase, leading to apoptosis	[16]
HCT-116, HeLa	*In vitro*	0.056, 0.112, 0.224, 0.448, 0.896, 1.792 mg/mL	It showed cell proliferation inhibitory activity against HCT-116 and HeLa cells *in vitro*	[17]
S-180, HT-29, HCT-116	*In vitro*	5 mg/mL	*In vitro*, it showed tumor cell proliferation inhibition on S-108 cells, HCT-116 and HT-29 cells	[18]
H22	*In vitro*	500 μg/mL	It destroys the cell microtubule network, arrests the cell cycle in G_2-M phase, and induces apoptosis	[19]
Male mice	A tumor-bearing mouse model was established by subcutaneous injection of H22 cells	50, 100, 200 mg/kg m_b	Inducing apoptosis in hepatocellular carcinoma cells by G_2-M phase arrest, increased ROS levels, down-regulation of Bcl-2 expression and activation of caspase-3 expression	[20]
CAF	*In vitro*	0, 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL	Inhibit the growth of prostate cancer cells and change the function of prostate CAF by activating TLR4-NF-κB pathway	[21]
CAF, PC-3	*In vitro*	0.5 mg/kg	Activating p21 expression in PC-3 cells by regulating the FoxO pathway induces G_0-G_1 cell cycle arrest in PC-3 cells	[22]
Male athymic nude mice	A mouse model of colon cancer was established by subcutaneous injection of HT-29 cells	0.2, 1, 5 mg/kg m_b	Apoptosis is induced by ROS-mediated endogenous and TNF-α-mediated exogenous pathways	[23]
Female athymic nude mice	A mouse model of breast cancer was established by subcutaneous injection of MCF-7 cells	1 mg/kg m_b	The proliferation of MCF-7 cells was inhibited and apoptosis was promoted by inhibiting the activation of PI3K-Akt-MDM2-p53 and PI3K-Akt-mTOR pathways and activating ERK-dependent pathways. Apoptosis of tumor cells was induced by activating caspase-3 signaling pathway	[24]
Mouse	A mouse model of colorectal cancer was established by subcutaneous injection of CT-26 cells	30 mg/kg m_b	Inhibiting lymphangiogenesis and lymphatic metastasis in colorectal cancer mice through the TLR4-JNK pathway of CAF cells,	[25]
HeLa	*In vitro*	5 mg/mL	The ratio of Bax/Bcl-2 was increased, mitochondrial membrane potential was lost, cytochrome c was released from mitochondria to cytoplasm, and caspase-9 and caspase-3 were activated. Mitochondrial-mediated signaling pathway was involved in LNT-induced apoptosis	[26]
Female mice	A tumor-bearing mouse model was established by subcutaneous injection of H22 cells	5 mg/kg m_b	Biodegradable LNT fragments released by macrophages promote the infiltration of macrophages, neutrophils and dendritic cells into tumors, and activate immune cells to enhance their cytotoxicity to tumor cells, resulting in tumor growth inhibition	[27]

Note: phosphoinositide-3 kinase-protein kinase B (PI3K-Akt); B-cell lymphoma-2 (Bcl-2); phase/mitotic period (G_2-M); reactive oxygen species (ROS); Toll-like receptor 4 (TLR4); cancer-associated fibroblast (CAF); forkhead box O (FoxO); nuclear factor-kappa B (NF-κB); murine double minute 2 (MDM2); mammalian target of rapamycin (mTOR).

induced oxidative stress and apoptosis in human umbilical vein endothelial cells (HUVEC) by modulating the ROS-p38-MAPK pathway, reverses type 1 diabetes by stimulating the production of regulatory T cells from $CD4^+$ T cells, reverses type 1 diabetes by activating the Nrf2- HO-1 signaling pathway to regulate streptozotocin (STZ)-induced type 2 diabetes (Table 2). Therefore, the study of the action mechanism of LNT in the treatment of diabetes, alleviation of diabetic complications, and protection of pancreatic cells is of potential research significance for the development of it as a therapeutic or adjunctive drug for the treatment of diabetes, as well as for the development of it as a nutraceutical product with a pancreas-protective effect.

Table 2 Recent studies on treatment of diabetes effect and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
HUVEC	HUVEC injury induced by high glucose	200, 400, 800 mg/L	Regulating the ROS-p38-MAPK pathway, enhance the autophagy level of HUVEC cells, and improve intracellular oxidative stress	[30]
HUVEC	*In vitro*	0.012 5, 0.025, 0.05 mg/mL	Inhibiting MAPK signaling pathway can reduce oxidative stress and apoptosis of HUVEC cells induced by high glucose, and effectively inhibit the formation of AGEs	[31]
Female non-obese diabetic mice	Intraperitoneal injection of LNT	5 mg/kg mb	LNT can stimulate $CD4^+$ T cells to produce regulatory T cells, which can be used to reverse hyperglycemia in early and late stage of type 1 diabetes	[32]
Pancreatic β cells	*In vitro*	0.012 5, 0.025, 0.05, 0.1, 0.2 mmol/L	Inhibition of MAPK and NF-κB and signaling pathways reduces glucose-induced oxidative stress and inhibits pancreatic β-cell apoptosis	[33]
MIN6	*In vitro*	0.012 5, 0.025, 0.05 mg/mL	Inhibiting p38-MAPK and JNK signaling pathway and activating Nrf2 signaling pathway; High glucose-induced apoptosis was prevented by reducing Bax expression and activating caspase-1 and caspase-3	[34]
Male mice	Type 2 diabetes model was established by intraperitoneal injection of STZ after high-fat and high-sugar feeding	50, 100, 200 mg/kg mb	The mRNA and protein expressions of Nrf2-HO-1 signaling pathway are up-regulated, and diabetes is treated by regulating oxidative stress	[35]

Note: nuclear factor-E2-related factor 2 (Nrf2); heme oxygenase 1 (HO-1); mitogen-activated protein kinase (MAPK); advanced glycation end products (AGEs); c-Jun N-terminal kinase (JNK).

2.3 Enteroprotective activity The gut microbiota plays a crucial role in host cell metabolism, pathogen resistance, and immune organ development and can be modified by dietary changes[36]. LNT was found to slow down rotavirus (RV)-induced diarrhea by improving ileal mucosal immune function, increasing antioxidant capacity, and decreasing apoptosis; ameliorate lipopolysaccharide (LPS)-stimulated jejunal cell death by modulating key genes of programmed necrosis, pyroptosis, and autophagy signaling pathways; and can shift the overall composition of the intestinal flora from thick-walled phylum-dominated to bacillus-mimetic phylum-dominated to regulate intestinal microbial dysbiosis; in addition, it can alter the NF-κB signaling pathway to improve the morphology of colon tissues and reduce the pro-inflammatory cytokines in antibiotic-induced intestinal microbial dysbiosis in mice (Table 3). Therefore, LNT is highly likely to be a natural agent for the treatment of bacterial viral intestinal damage and may be a good prebiotic supplement to protect the gut by improving the composition of the intestinal flora.

Table 3 Recent studies on intestinal protection effect and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
Pig	Feeding RV solution made piglet diarrhea.	84 mg/kg m_b	Improve the ileal mucosal immune function of piglets, thereby alleviating RV infection	[37]
Weanling pig	Peritoneal injection of LPS-stimulated piglet jejunum cell death	0.02%	Maintain intestinal integrity by regulating key genes in programmed necrosis, pyroptosis, and autophagy signaling pathways	[38]
Mouse	The intestinal flora of LNT and normal fed mice were compared	40 mg/kg m_b	The overall composition of intestinal flora in mice changed from Firmicutes to Bacteroidetes	[39]
Weanling pig	Piglet viral diarrhea caused by oral RV	0, 84 mg/kg m_b	RV-induced diarrhea in piglets was alleviated by increasing antioxidant capacity, reducing apoptosis, increasing microbiota and improving intestinal barrier	[40]
Male mice	Antibiotic-induced intestinal microbial dysbiosis in mice	200 mg/kg m_b	By regulating the intestinal flora, the expression of NF-κB signaling pathway was down-regulated, the expression of tight junction protein was increased, the level of pro-inflammatory cytokines was decreased, and the content of short-chain fatty acids was increased	[41]

2.4 Anti-inflammatory activity Inflammation is a natural protective response of the innate immune system in response to injury or external stimuli such as allergens, pathogens, infections, and irritants[42-43]. Studies have shown that LNT has good anti-inflammatory activity, and it can inhibit high-fat diet-induced adipose tissue inflammation, Klebsiella-induced pneumonia, 2,4-dinitrochlorophenyl ethanol and dextran sodium sulfate (DSS)-induced colitis, LPS-induced mastitis, and septic shock, *etc.* The mechanism of LNT anti-inflammatory activity is mainly as follows: inhibiting the RIPK1-RIPK3-MLKL signaling pathway, regulating the activation of NOD-like receptor thermal protein domain associated protein 3 (NLRP3) and inhibiting the activation of the Wnt-β-catenin pathway. The therapeutic effect of LNT on inflammation is multi-targeted, and it can treat a variety of inflammatory responses induced by different modalities, but the relevant experiments are cell or animal model experiments, and there is a lack of relevant clinical trial data, so the mechanism of anti-inflammatory activity of LNT can be further investigated, to provide theoretical support for its use as a clinical trial, which is expected to become a new natural anti-inflammatory drug. The action mechanism and effect of the anti-inflammatory activity of LNT are shown in Table 4.

Table 4 Recent studies on anti-inflammatory effect and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
Male mice	Obese mouse model was established by high-fat diet	500 mg/kg m_b	Inhibiting adipose tissue inflammation in high-fat diet mice and improving glucose and lipid metabolism disorders in adipose tissue	[30]
Bovine mammary epithelial cells	*In vitro*	0, 50, 100, 200, 300 μg/mL	By regulating the Nrf2 pathway to exert anti-inflammatory, antioxidant and anti-apoptotic effects, thereby reducing LPS-induced bovine mammary epithelial cell injury	[44]
Male rat	Pulmonary sepsis model was established by intratracheal administration of Klebsiella pneumoniae	20 mg/kg m_b	Reducing bacterial count and white blood cell infiltration into the lungs during pneumonia; Improving lung function and circulating metabolite levels; decreased IL-10 and increased TNF-α levels enhanced the inflammatory response to infection	[45]
Female mice	DSS induced colitis model in mice	2 mg/mL m_b	PIK1-RIPK3-MLKL necrosis signal was significantly inhibited by PIK1-RIPK3-MLKL necrosis signal, resulting in decreased phosphorylated MLKL levels in the colon of colitis mice	[46]
A549	*In vitro*	100 μg/mL	It inhibits caspase-1 activity, down-regulates the expression of IL-6 and IL-1β, and increases ROS and mitochondrial dysfunction. Its level does not induce cell death, but regulates NLRP3 activation	[47]
Female mice	DSS induced colitis model in mice	100 μg/μL m_b	The ileum regulates a balanced Th1 immune response and then inhibits colitis	[48]
Mouse	Mastitis model was established by abdominal mammary injection of LPS	5, 10, 20 mg/kg m_b	The anti-inflammatory effect is exerted by inhibiting the activation of Wnt-β-catenin pathway	[49]
SW1353	*In vitro*	250 – 500 μg/mL	By reducing the expression of COX-2 and iNOS in SW1353 cells, the production of pro-inflammatory cytokines was inhibited. Reducing the activation of NF-κB signaling pathway	[50]
Male mice	The septic shock model was established by intraperitoneal injection of LPS	2, 10 mg/kg m_b	Selective inhibition of IL-1β maturation induced by AIM2 inflammasome activation	[51]

Note: receptor-interacting protein kinase 1-receptor-interacting protein kinase 3-mixed lineage kinase domain-like (RIPK1-RIPK3-MLKL); interleukin-10 (IL-10); tumor necrosis factor-α (TNF-α); cyclooxygenase-2 (COX-2); inducible nitric oxide synthase (iNOS); absent in melanoma 2 (AIM2).

2.5 Antioxidant and anti-aging activity Oxidative stress, an adverse reaction caused by ROS, is a major factor contributing to aging and various age-related diseases in humans and other animals[52]. Studies have shown that LNT possesses antioxidant and anti-aging activities. LNT protects against H_2O_2, benzo(a)pyrene-induced damage to human immortalized keratinocytes (HaCaT); improves the antioxidant capacity of loach under ammonia-nitrogen stress; extends the lifespan of *Cryptobacterium hidradii* nematodes and protects the nematodes by its antioxidant capacity from oxidative stress; and reversing age-altered gut microbiota structure. In addition, the antioxidant capacity of LNT may have applications in the field of sperm cryopreservation. During processes, such as freezing and thawing of semen, ice crystal formation, osmotic imbalance, and oxidative stress can occur, causing irreversible damage to sperm[53]. In contrast, LNT can reduce malondialdehyde (MDA) content in semen, elevate total antioxidant capacity (T-AOC) and total glutathione (T-GSH), activate spermatozoa's own antioxidant system, and improve sperm viability and acrosomal integrity after thawing of frozen spermatozoa. Sperm viability and acrosome integrity after thawing of frozen spermatozoa[54]. The action mechanism and effect of LNT antioxidant and anti-aging activities are shown in Table 5.

Table 5 Recent studies on anti-oxidation and anti-aging effect and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
Loach	LNT is added to the feed and fed	1.0 mg/g	LNT can improve the antioxidant capacity of Misgurnus anguillicaudatus under ammonia nitrogen stress	[55]
HaCaT	H_2O_2 induced cell damage	7.5, 15, 30, 60 μg/mL	LNT can enhance the tolerance and anti-stress of cells to oxidative damage, and has protective and repair effects on damaged cells	[56]
Caenorhabditis elegans	Culturing with buffer solution	0.05, 0.25, 1.25 mg/mL	By enhancing the nuclear translocation of DAF-16 and SKN-1, the lifespan of *C. elegans* is prolonged and *C. elegans* is protected from oxidative stress	[57]
HaCaT	Benzopyrene induced cell damage	7.5, 15, 30, 60 μg/mL	By reducing the content of MDA and increasing the activity of SOD and GSH-Px, the redox balance of cells was restored	[58]
Male mice	Elderly mice were given LNT by gavage	40 mg/kg m_b	Increase the level of peripheral blood cytokines to restore the age-attenuated immune response and reverse the age-changed intestinal microflora structure	[59]

2.6 Hepatoprotective activity The liver is a major metabolic organ in vertebrates and other animals, responsible for primary detoxification, and has a variety of functions in the body, including glycogen storage and catabolism, erythrocyte catabolism, phagocytosis, and detoxification. Therefore, the liver is susceptible to damage caused by various toxins or metabolites[60-62]. Studies have shown that LNT has hepatoprotective activity and has therapeutic effects on high-fat-induced nonalcoholic fatty liver disease, sodium arsenite-induced hepatotoxicity, and LPS-induced liver injury, and its action mechanism includes activation of the PPARα pathway, reduction of oxidative stress and apoptosis, and participation in tumor necrosis factor receptor superfamily member 4 (OXF4), which is the most effective and efficient way to prevent liver damage. Superfamily member 4 (OX40) or IL-17A down-regulation, activation of Nrf2 and quinone oxidoreductase 1 (NQO1) signaling pathways can enhance antioxidant capacity and inhibit lipid peroxidation progression (Table 6). Therefore, LNT can be utilized to develop into nutraceuticals with hepatoprotective effects or natural preparations for clinical treatment of liver injury.

Table 6 Recent studies on hepatoprotective effect and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
Male mice	Establishment of nonalcoholic fatty liver disease model by high fat diet	6 mg/kg m_b	It can improve hepatic steatosis, reduce oxidative stress and apoptosis by activating PPARα pathway	[63]
Male mice	A mouse model of liver toxicity was established by intragastric administration of sodium arsenite	1.0 mg/kg m_b	It is involved in the down-regulation of OX40 or IL-17A and the activation of antioxidant Nrf2 and NQO1 signals, and antagonizes sodium arsenite-induced hepatotoxicity in mice	[64]
Male mice	Liver injury model was established by intraperitoneal injection of LPS	200, 400, 600 mg/kg m_b	LNT increased the activities of SOD, CAT, GSH-Px and T-AOC, decreased the contents of MDA and LPO, and exerted hepatoprotective activity by inhibiting the progression of lipid peroxidation	[65]

Note: catalase (CAT); glutathione peroxidase (GSH-Px); lipid peroxidation (LPO).

2.7 Immunomodulatory activity In recent years, immunotherapy has attracted the attention of many researchers, which can protect the organism from cancer by stimulating immune effector cells such as, lymphocytes, macrophages, neutrophils, natural killer cells, and dendritic cells to target abnormal antigens expressed on the surface of tumor cells[66]. In addition, oral administration of some bioactive macromolecules induces intestinal mucosal immunity, a primary response that is extremely important in the induction of immune tolerance and innate immunity against intracellular pathogens[67-69].

Studies have shown that LNT has immunomodulatory activity, and the mechanisms by which LNT exerts its immunomodulatory activity in *in vitro* cellular assays are: (i) by regulating the decrease in the level of chemokine ligand-2 (CCL-2); (ii) by regulating the MAPK signaling pathway. The mechanisms of action in animal studies are: (i) immunosuppressive mouse model of cyclophosphamide (CTX), up-regulating the proportion of activated T cells, the number of M cells in Peyer's patches (PPs) and their enhanced antigen-transferring ability; and (ii) mouse model of S-180 tumors, which induces NO production, activation of macrophage phagocytosis, induction of lymphocyte proliferation, and up-regulation of $CD4^+$ T cell levels in lymphoid organs; (iii) a mouse model of breast cancer, by decreasing the expression of IL-35, increasing the secretion of interferon-γ (IFN-γ), and promoting the cellular activity of CD4 Th, and T helper cell 17 (Th17); (iv) a mouse model of breast cancer in Normal mice, which significantly enhanced the immune response by affecting gene expression in the small intestine, cecum, and colon (Table 7). In addition, it has been shown that covalent attachment of LNT to multi-walled carbon nanotubes enhances the immunoreactivity of LNT, and that the nanotube structure allows rapid access to dendritic cells and induces phenotypic and functional maturation of dendritic cells[70]. Therefore, the immunomodulatory activity of LNT can be fully utilized to develop it to be a health food with immune-enhancing effects or a therapeutic drug for clinical immunotherapy.

Table 7 Recent studies on immune-regulating effect and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
HaCaT	Benzopyrene induced cell damage	7.5, 15, 30, 60 μg/mL	The levels of IL-8 and CCL-2 decreased, indicating that LNT reduces inflammation and plays an immunoregulatory role	[58]
Female mice	Immunosuppressive mouse model was established by injection of CTX	400, 600, 800 mg/kg m_b	The percentage of T cells and B lymphocytes was maintained, and the proportion of activated T cells was up-regulated. The number of M cells and their antigen transfer ability were enhanced	[71]
RAW264.7	*In vitro*	12.5, 50, 100 μg/mL	It plays an immune-enhancing role by activating the MAPK signaling pathway	[72]
Male mice	S-180 cells were injected into the ascites of mice to establish a tumor-bearing mouse model	100 mg/kg m_b	It exerts immunomodulatory activity by inducing NO production, macrophage phagocytosis and lymphocyte proliferation involved in anti-tumor activity	[73]
Female mice	A mouse model of breast cancer was established by injecting breast cancer 4T1 cells	200 mg/kg m_b	It can reduce the expression of IL-35 in tumor tissue and spleen tissue, increase the secretion of IFN-γ, reduce the inhibitory activity of T cells and other cells *in vivo*, and promote the activity of CD4 Th, Th17 and other cells	[74]
Female mice	Tumor-bearing mouse model was established by intraperitoneal injection of S-180 cells	1 mg/kg m_b	Up-regulating the level of $CD4^+$ T cells in lymphoid organs, reducing tumor load and promoting immune regulation	[75]
Male mice	LNT was administered to mice by gavage	40 mg/kg m_b	The immune response is significantly enhanced by affecting gene expression in the small intestine, cecum and colon	[76]

2.8 Other activities In addition to anticancer, diabetes treatment, intestinal protection, anti-inflammatory, antioxidant, anti-aging, hepatoprotective, and immunomodulatory activities, LNT also has antirespiratory infections, protection against lung and kidney damage, enhancement of bone metabolism, antiviral, alleviation of depression, alleviation of myelosuppression, neuroprotection, and improvement of cognitive disorders, and its action mechanism and effects are shown in Table 8. When LNT and epirubicin are used in combination, LNT has a direct potentiating effect on the killing of bladder cancer cells by epirubicin[77]. In addition, a study added LNT to the feed of European eel, which promoted the growth of European eel, and also lowered blood lipids and improved liver health[78]; LNT was used as a seed dressing to plant wheat seeds, which effectively prevented and controlled wheat sharp eye spot[79]. In addition, in a study, researchers conducted an interesting experiment to utilize the property that the chemical and nutrient composition of shiitake mushrooms change significantly when they are grown on different substrates, and researchers replaced the traditional shiitake mushroom medium with astragalus to obtain a kind of astragalus shiitake mushroom, and the study showed that this LNT had better *in vitro* anti-proliferative activity against colon cancer HCT-116 cells[80].

3 Structure-activity relationship of LNT

LNT is a glucan molecule with β-D-(1→3)-Glc as the main chain and 1→6 and 1→3 linked glucose residues as the side chains[95]. The bioactivity of LNT is closely related to its spatial structure, molecular mass, and molecular composition, and in addition, chemical modifications have a greater impact on the bioactivity of LNT. Therefore, it is valuable to explore the structure-activity relationship of LNT to elucidate how its complex structure exerts its biological activity.

3.1 Spatial structure and molecular mass of LNT in relation to its activity Zou *et al*[75] extracted polysaccharides from shiitake mushrooms of three different periods, autumn-grown mushrooms (AG), spring-grown mushrooms (SG) and cultivated-grown mushrooms (CG), AG and SG existed in a network-like form, in contrast to CG which did not form a network but existed as random aggregates, and AG which was in a network-like form showed a higher tumor-inhibitory activity than CG, which did not form a network-like form. Wang *et al*.[96] isolated five types of LNTs and studied them and found that LNTs with high molecular weights (617.6 and 638.7 kDa) were more effective in inhibiting the proliferation of tumor cells than the other ones with low molecular weights (97.57, 273.8 and 151.3 kDa.) Zhao *et al*.[17, 87] and Zhang *et al*.[20] showed that LNT with molecular weight between 200 and 800 kDa had better tumor cell proliferation inhibitory activity. As a natural macromolecular compound, the spatial structure and molecular weight of LNT are two important evaluation indexes. LNT with network shape and high molecular weight is more advantageous in anti-tumor cell proliferation inhibitory activity.

3.2 Relationship between the molecular composition of LNT and its activity Shiitake mushroom has a strong ability to absorb metal ions in the medium, and the extracted LNT often contains various metal ions such as, Mg, K, Ga, Zn, *etc*. Qian *et al*[97] removed the metal ions from LNT and obtained deionized LNT to explore the activity of LNT before and after deionization, and the study showed that, after removing the intrinsic metal ions, the antioxidant capacity of polysaccharides was reduced, and their inhibitory effect on the tumor cell proliferation was inhibition of tumor cell proliferation was weakened. Therefore, the metal ions present in LNT are its important components, and its antioxidant capacity and anti-tumor proliferation activity are closely related to the metal ions.

Table 8 Recent studies on other biological activities and action mechanism of LNT

Subjects	Method	Dosage	Mechanism and effect	Reference
Human bladder cancer cells	*In vitro*	LNT:100 nmol/L; 2.5, 5.0, 10 μmol/L	LNT has a direct synergistic effect on epirubicin, which is independent of the up-regulation of inflammatory factors TNF-α and IL-6	[77]
Anguilla anguilla	Added to the feed	480 mg/kg	Promoting the growth of European eel, reducing blood lipids, and improving liver health	[78]
Wheat seed	LNT was used as seed dressing agent to plant wheat seeds	40, 80 mg/kg	The transcription of AOX, β-1, 3-glucanase, salicylic acid signaling pathway related gene NbPR1a and sharp eye spot resistance related gene RS33 was increased	[79]
Male mice	A mouse model of lung cancer was established by intratracheal instillation of $PM_{2.5}$ after tail vein injection of A549 cells	3 mg/kg m_b	The combination of potential probiotics C17 and D19 strains with LNT can regulate the diversity of respiratory flora in lung cancer mice exposed to $PM_{2.5}$	[81]
Male mice	Sepsis model was established by cecal ligation and perforation method	100 mg/kg m_b	It may play a protective role in mice with sepsis-induced lung injury by inhibiting mTOR pathway and activating autophagy	[82]
Mouse	A mouse model of renal injury was established by tail vein injection of cisplatin	0, 5, 10, 20, 40, 60, 80, 100 mg/kg m_b	The accumulation of ROS was prevented by activating Nrf2-ARE signaling pathway to alleviate cisplatin-induced nephrotoxicity	[83]
Male mice	Acute lung injury model was established by tail vein injection of LPS	50, 100 mg/kg m_b	By inhibiting IL-6, IL-1β and NO signaling molecules, and down-regulating the expression of iNOS, COX-2, TLR4 and NF-κB p65 proteins, it plays an anti-inflammatory role and has a protective effect on sepsis-induced acute lung injury	[84]
Rat	A rat model of vitamin D deficiency was established by feeding rats with vitamin D-deficient diet	150, 450 mg/kg m_b	By acting on the vitamin D-FGF-23-Klotho axis, the axis factor is regulated, the content of vitamin D is increased, and the osteogenic disorder caused by vitamin deficiency is improved	[85]
Female mice	A model of avian influenza virus infection was established by inoculating H5N1 strain	500 μg/mL m_b	Combined with the immunostimulatory activity of LNT and the drug antigen delivery ability of $CaCO_3$, $CaCO_3$-LNT-H5N1 can induce stronger cellular and humoral immune responses	[86]
HepG2.2.15	Infection with HBV virus	10, 20, 30, 40, 50 mg/mL	LNT has strong anti-HBV activity *in vitro*	[87]
Mouse	Chronic stress depression model in mice induced by unpredictable stress stimulation	2.5, 5.0 mg/kg m_b	LNT can increase SOD, antagonize MDA content, alleviate depressive symptoms in chronic stress model mice, and increase the autonomic activity time of mice	[88]
Macrophages	*In vitro*	10, 20, 40 μg/mL	LNT induces the production of G-CSF, GM-CSF and M-CSF by activating the MAPK/NF-κB signaling pathway in cells, thereby alleviating therapeutic erythromycin-induced bone marrow suppression	[89]
Epithelioma papulosum cyprini (EPC)	*In vitro*	0.56, 3.12, 6.25, 12.5, 25, 50, 100 μg/mL	After IHNV attack, the expression levels of TNF-α, IL-2 and IL-11 were significantly down-regulated, and the expression levels of IFN-1 and IFN-γ were up-regulated. The mechanism may be related to the regulation of innate immune response and specific immune regulation	[90]
Male mice	To establish a mouse model of central nervous system injury induced by Toxoplasma gondii infection	1 mg/kg m_b	By reducing the entry of $CD8^+$ T cells into the CNS through the blood-brain barrier, the extensive inflammatory response of the nervous system is reduced; by reducing the number of $CD8^+$ IFN $-\gamma^+$ T cells in the brain tissue, regulating the secretion level of neurotransmitters, and improving the inflammatory response in the mouse brain	[91]
Male mice	Partial hepatectomy model in aged mice was established by removing part of liver tissue	20 mg/kg m_b	LNT inhibits the release of inflammatory cytokines TNF-α, IL-1β and IL-6 in peripheral blood and hippocampus, and can effectively improve postoperative cognitive dysfunction in elderly mice with partial hepatectomy	[92]
Male mice	A mouse model of obese cognitive impairment was established by high-fat diet-induced obesity	500 mg/kg m_b	Increased mucosal thickness in mice fed a high-fat diet, up-regulated the expression of tight junction protein occludin, reduced plasma LPS levels, and inhibited the accumulation of pro-inflammatory macrophages in the colon	[93]
Male mice	Dicyclohexanone oxalyl dihydrazone was added to the feed to establish a mouse model of acute demyelination with regenerable myelin sheath	4, 10, 20 mg/kg m_b	It promotes the transformation of microglia from M_1 to M_2 state, enhances IL-10 and BDNF, inhibits TNF-α and IL-1β, and down-regulates microglia activation, oligodendrocyte and astrocyte proliferation by regulating dectin-1 to regulate neuroimmune imbalance	[94]

Note: antioxidant-response element (ARE); Hepatitis B (HBV); Infectious hematopoietic necrosis virus (IHNV); central nervous system (CNS); alternative oxidase (AOX); brain-derived neurotrophic factor (BDNF).

3.3 Effect of chemical modifications on LNT bioactivity

Kaleta *et al*[98] and Klimaszewska *et al*[99] found that LNT selenated had stronger antioxidant activity and immunostimulatory activity, and that selenated LNT increased cell viability and enhanced cytotoxicity against cancer cells. Wang *et al*[100] and Wang J *et al*[101] found that sulphated LNT, compared with unsulphated, possessed stronger antibacterial activity, antiviral infection activity and inhibition of tobacco mosaic virus proliferation activity in a dose-dependent manner. Wang *et al*[102] found that zincation-modified LNT possessed stronger anti-aging activity and *in vitro* antioxidant activity. In conclusion, different ways of chemical modification can have a large impact on the biological activity of LNT. In contrast to unmodified LNT, its derivatives tend to have more biological activities or enhanced on the basis of existing activities. Therefore, exploring the effect of modifying groups on the activity of LNT can provide a theoretical basis for the development of LNT derivatives with specificity.

4 Conclusions

LNT has been used clinically in adjuvant cancer treatment and postoperative tumor therapy, and related nutraceuticals and injections have appeared. However, compared to its many biological activities (*e. g.*, anti-inflammatory, antioxidant, and intestinal protection, *etc.*), LNT still has great value for development and utilization. Since the discovery of LNT, there have been many studies on its biological activity, action mechanism and molecular structure. However, due to the influence of many factors, such as the extraction method and the mushroom substrate itself, the molecular weights of the different components of LNT are irregular, the molecular structure is complex, the efficacy of the different levels of action, and the relationship between the structure and the bioactivity is not easy to conclude, which has constrained the in-depth study of LNT. It is difficult to have a unified standard for the evaluation of LNT, which is also the reason why most countries prohibit the use of LNT in clinical practice.

In view of the existing problems of LNT, the research on LNT can be started from the following aspects: (i) On the basis of the existing studies, the multilevel structures of different components of LNT can be clarified, and the connection between its structure and biological activity can be deeply investigated. (ii) For chemically modified LNT, the influence of the modifying groups on the structure and biological activity of LNT can be investigated at the molecular level. (iii) It has been reported that the combination of LNT with other drugs can enhance the therapeutic effect of the drug, and the activity of the vaccine can be enhanced. LNT can enhance the therapeutic effect of drugs and the activity of vaccines. Therefore, the action mechanism of LNT when used as a supplement can be explored to fully investigate the bioactivity of LNT. (iv) According to a few studies, it has been reported that changing the medium of shiitake mushrooms can change the bioactivity of LNT, for example, when shiitake mushrooms are cultured on astragalus, the LNT obtained has more significant antiproliferative activity, therefore, the change of the medium can be investigated for its influence on LNT bioactivity. Therefore, it is possible to investigate the effect of the change of culture medium on the biological activity of LNT, which will provide new ideas on how to obtain LNT with better biological activity. (v) With the development of the society, people pay more attention to the green and natural products that are beneficial to human health, and the development of LNT extracted from shiitake mushrooms, which is a medicinal and food component, into health care products, functional animal feeds, and plant seed dressings will be the trend of the development in the future. In conclusion, the research on LNT still needs to be supplemented by a large number of more in-depth studies.

References

[1] WANG J T, WANG H C, LIU L. Progress in research on structure-function relationship of lentinan[J]. Food Science, 2019, 40(19): 363 – 369. (in Chinese).

[2] JIANG T, LUO S, CHEN Q, *et al.* Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinula edodes*)[J]. Food Chemistry, 2010, 122(3): 761 – 767.

[3] LI YM. Research status and prospect of *Lentinula edodes*[J]. Microbiology China, 2005(4): 149 – 152. (in Chinese).

[4] MATTILA P, KÖNKÖ K, EUROLA M, *et al.* Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms[J]. Journal of Agricultural and Food Chemistry, 2001, 49(5): 2343 – 2348.

[5] CHIHARA G, MAEDA Y, HAMURO J, *et al.* Inhibition of mouse sarcoma 180 by polysaccharides from *Lentinula edodes* (Berk.) sing[J]. Nature, 1969, 222(5194): 687 – 688.

[6] GYAMFI J, KIM J, CHOI J. Cancer as a metabolic disorder[J]. International Journal of Molecular Sciences, 2022, 23(3): 1155.

[7] SUNG H, FERLAY J, SIEGEL R L, *et al.* Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. Ca-a Cancer Journal for Clinicians, 2021, 71(3): 209 – 249.

[8] LI B, SHAO H, GAO L, *et al.* Nano-drug co-delivery system of natural active components and chemotherapy drugs for cancer treatment: A review[J]. Drug Delivery, 2022, 29(1): 2130 – 2161.

[9] YI J, LI Q, SUN LD, *et al.* Research advancement in natural anti-cancer product[J]. China Journal of Chinese Materia Medica, 2019, 44(1): 19 – 27. (in Chinese).

[10] HUANG Z, LI YZ, ZHAO WY, *et al.* Effects of lentinan combined with gemcitabine on the biological behavior of human pancreatic cancer cell line ASPC-1[J]. Journal of China Medical University, 2020, 49(3): 215 – 219. (in Chinese).

[11] CHEN WX, WEI XM, WANG CH. Inhibitory effect of lentinan on tumor growth in thyroid cancer-bearing mice[J]. The Chinese Journal of Clinical Pharmacology, 2019, 35(22): 2875 – 2877. (in Chinese).

[12] WAN XL, REN YH, LU RN, *et al.* Effects of lentinan on proliferation, cell cycle, apoptosis and migration of glioma SHG-44 cells[J]. Chinese Traditional Patent Medicine, 2019, 41(11): 2614 – 2619. (in Chinese).

[13] MA L, RONG F, WANG JB, *et al.* Effect of lentinan on apoptosis and PI3K/AKT signaling pathway in leukemic HL-60 cells *in vitro*[J]. Chinese Journal of Pathophysiology, 2019, 35(6): 1069 – 1074. (in Chinese).

[14] RUI W, YOU SY, WU XQ, *et al.* Effect of Letinous edodes polysaccharide on expression of immunogenic cell death related molecules in human colorectal cancer epithelial SW837 cell line[J]. Chinese Journal of Immunology, 2019, 35(8): 948 – 952. (in Chinese).

[15] ZHANG QL, DU ZS, WANG KP, *et al.* Molecular mechanism of *Lentinula edodes* polysaccharide to induce tumor cell apoptosis by inhibiting Akt pathway ang tubulin polymerization[J]. Chinese Journal of Hospital Pharmacy, 2016, 36(23): 2046 – 2051. (in Chinese).

[16] YOU RX, ZHONG Y, WANG L, *et al.* Preliminary study on the apoptosis mechanisms of mouse hepatocarcinoma H22 cells induced by polysac-

charides derived from *Lentinula edodes*[J]. Chinese Journal of Hospital Pharmacy, 2015, 35(9): 776 – 781. (in Chinese).
[17] ZHAO Y M, WANG J, WU ZG, *et al.* Extraction, purification and anti-proliferative activities of polysaccharides from *Lentinula edodes*[J]. International Journal of Biological Macromolecules, 2016(93): 136 – 144.
[18] JEFF IB, YUAN X, SUN L, *et al.* Purification and *in vitro* anti-proliferative effect of novel neutral polysaccharides from *Lentinula edodes*[J]. International Journal of Biological Macromolecules, 2013(52): 99 – 106.
[19] YOU R X, LIU J Y, LI SJ, *et al.* Alkali-soluble polysaccharide, isolated from *Lentinula edodes*, induces apoptosis and G_2/M cell cycle arrest in H22 cells through microtubule depolymerization[J]. Phytotherapy Research, 2014, 28(12): 1837 – 1845.
[20] ZHANG Q, DU Z, ZHANG Y, *et al.* Apoptosis induction activity of polysaccharide from *Lentinula edodes* in H22-bearing mice through ROS-mediated mitochondrial pathway and inhibition of tubulin polymerization [J]. Food & Nutrition Research, 2020: 64.
[21] XU Y, MA J, ZHENG Q, *et al.* MPSSS impairs the immunosuppressive function of cancer-associated fibroblasts via the TLR4-NF-kappaB pathway[J]. Bioscience Reports, 2019, 39(5): 1 – 10.
[22] ZHANG T, CHEN X, SUN L, *et al.* Proteomics reveals the function reverse of MPSSS-treated prostate cancer-associated fibroblasts to suppress PC-3 cell viability via the FoxO pathway[J]. Cancer Medicine, 2021, 10(7): 2509 – 2522.
[23] WANG J, LI W, HUANG X, *et al.* A polysaccharide from *Lentinula edodes* inhibits human colon cancer cell proliferation and suppresses tumor growth in athymic nude mice[J]. Oncotarget, 2017, 8(1): 610 – 623.
[24] XU H, ZOU S, XU X. The β-glucan from *Lentinula edodes* suppresses cell proliferation and promotes apoptosis in estrogen receptor positive breast cancers[J]. Oncotarget, 2017, 8(49): 86693 – 86709.
[25] WANG Y, ZENG Y, ZHU L, *et al.* Polysaccharides from *Lentinula edodes* inhibits lymphangiogenesis via the Toll-Like receptor 4/JNK pathway of cancer-associated fibroblasts[J]. Frontiers in Oncology, 2021 (10): 547683.
[26] YA G. A *Lentinula edodes* polysaccharide induces mitochondrial-mediated apoptosis in human cervical carcinoma HeLa cells[J]. International Journal of Biological Macromolecules, 2017(103): 676 – 682.
[27] ZHENG X, ZHOU F, XU X, *et al.* Uptake of intraperitoneally administrated triple helical beta-glucan for antitumor activity in murine tumor models[J]. Journal of Materials Chemistry B, 2017, 5(47): 9337 – 9345.
[28] ZHENG Y, LEY SH, HU FB. Global aetiology and epidemiology of type 2 diabetes mellitus and its complications[J]. Nature Reviews Endocrinology, 2018, 14(2): 88 – 98.
[29] GE D, SU BB, CHEN ZZ, *et al.* Correlation of NLR, PLR, MLR and vascular disease in Type 2 diabetes mellitus[J]. Journal of Medical Research, 2022, 51(7): 161 – 165. (in Chinese).
[30] YANG QW, NI HY, WU L, *et al.* The protective effect of lentinan regulating autophagy on HUVEC injury induced by high glucose and its mechanism[J]. Chinese Pharmacological Bulletin, 2022, 38(8): 1182 – 1189. (in Chinese).
[31] CAO X, XIA Y, LIU D, *et al.* Inhibitory effects of *Lentinula edodes* mycelia polysaccharide on alpha-glucosidase, glycation activity and high glucose-induced cell damage[J]. Carbohydrate Polymers, 2020(246): 116659.
[32] WU T, CAI Z, NIU F, *et al.* Lentinan confers protection against type 1 diabetes by inducing regulatory T cell in spontaneous non-obese diabetic mice[J]. Nutrition & Diabetes, 2023, 13(1): 4.
[33] CAO XY, LIU D, BI RC, *et al.* The protective effects of a novel polysaccharide from *Lentinula edodes* mycelia on islet beta (INS-1) cells damaged by glucose and its transportation mechanism with human serum albumin[J]. International Journal of Biological Macromolecules, 2019 (134): 344 – 353.
[34] CAO X, LIU D, XIA Y, *et al.* A novel polysaccharide from *Lentinula edodes* mycelia protects MIN6 cells against high glucose-induced damage via the MAPKs and Nrf2 pathways[J]. Food & Nutrition Research, 2019: 63.
[35] GONG P, WANG X, LIU M, *et al.* Hypoglycemic effect of a novel polysaccharide from *Lentinula edodes* on STZ-induced diabetic mice via metabolomics study and Nrf2/HO-1 pathway[J]. Food & Nutrition Research, 2022, 13(5): 3036 – 3049.
[36] WANG XY, WANG WJ, WWANG LM, *et al.* Lentinan modulates intestinal microbiota and enhances barrier integrity in a piglet model challenged with lipopolysaccharide[J]. Food & Function. 2019. 10(1): 479 – 489.
[37] MAO XB, HU HY, CHEN DW, *et al.* Effects of dietary lentinan on ileal barrier function of weaned pigs challenged by rotavirus[J]. Chinese Journal of Animal Nutrition, 2021, 33(8): 4323 – 4331. (in Chinese).
[38] LIANG TZ, WANG LM, YU C, *et al.* Effects of lentinan on jejunal cell death signaling pathway related gene expression of piglets challenged with lipopolysaccharide[J]. Chinese Journal of Animal Nutrition, 2020, 32 (2): 915 – 924. (in Chinese).
[39] XU X, ZHANG X. *Lentinula edodes*-derived polysaccharide alters the spatial structure of gut microbiota in mice[J]. Plos One, 2015, 10(1): e0115037.
[40] MAO X, HU H, XIAO X, *et al.* Lentinan administration relieves gut barrier dysfunction induced by rotavirus in a weaned piglet model[J]. Food & Function, 2019, 10(4): 2094 – 2101.
[41] JI X, SU L, ZHANG P, *et al.* Lentinan improves intestinal inflammation and gut dysbiosis in antibiotics-induced mice[J]. Surface Science Reports, 2022, 12(1): 19609.
[42] PHULL AR, MAJID M, HAQ IU, *et al.* *In vitro* and *in vivo* evaluation of anti-arthritic, antioxidant efficacy of fucoidan from *Undaria pinnatifida* (Harvey) Suringar[J]. International Journal of Biological Macromolecules, 2017(97): 468 – 480.
[43] JOSEPH SV, EDIRISINGHE I, BURTON-FREEMAN BM. Fruit polyphenols: A review of anti-inflammatory effects in humans[J]. Critical Reviews in Food Science and Nutrition, 2016, 56(3): 419 – 444.
[44] MENG M, HUO R, WANG Y, *et al.* Lentinan inhibits oxidative stress and alleviates LPS-induced inflammation and apoptosis of BMECs by activating the Nrf2 signaling pathway[J]. International Journal of Biological Macromolecules, 2022(222): 2375 – 2391.
[45] MASTERSON CH, MURPHY EJ, GONZALEZ H, *et al.* Purified beta-glucans from the Shiitake mushroom ameliorates antibiotic-resistant Klebsiella pneumoniae-induced pulmonary sepsis[J]. Letters in Applied Microbiology, 2020, 71(4): 405 – 412.
[46] ALAGBAOSO CA, MIZUNO M. Polysaccharides from shiitake culinary-medicinal mushroom *Lentinula edodes* (Agaricomycetes) suppress pMLKL-mediated necroptotic cell death and colitis in mice[J]. International Journal of Medicinal Mushrooms, 23(7): 13 – 26.
[47] LI M, DU X, YUAN Z, *et al.* Lentinan triggers oxidative stress-mediated anti-inflammatory responses in lung cancer cells[J]. Molecular And Cellular Biochemistry, 2022, 477(2): 469 – 477.
[48] MINATO KI, OURA K, MIZUNO M. The inhibitory effect of oral administration of lentinan on DSS-induced inflammation is exerted by the migration of T cells activated in the ileum to the colon[J]. European Journal Of Pharmacology, 2023(946): 175631.
[49] YIN H, XUE G, DAI A, *et al.* Protective effects of lentinan against lipopolysaccharide-induced mastitis in mice[J]. Frontiers in Pharmacology, 2021(12): 755768.
[50] ZHANG Z, ZHA Z, ZHAO Z, *et al.* Lentinan inhibits AGE-induced inflammation and the expression of matrix-degrading enzymes in human chondrocytes[J]. Drug Design Development and Therapy, 2020(14): 2819 – 2829.
[51] AHN H, JEON E, KIM JC, *et al.* Lentinan from shiitake selectively attenuates AIM2 and non-canonical inflammasome activation while inducing pro-inflammatory cytokine production[J]. Scientific Reports, 2017, 7(1): 1314.
[52] PISOSCHI A M, POP A. The role of antioxidants in the chemistry of oxidative stress: A review[J]. European Journal of Medicinal Chemistry, 2015(97): 55 – 74.

[53] TIWARI A, TEKCAN M, SATI L, *et al.* A new media without animal component for sperm cryopreservation: motility and various attributes affecting paternal contribution of sperm[J]. Journal of Assisted Reproduction and Genetics, 2017, 34(5): 647 – 657.
[54] GUO LY, CAO F, LI ZQ, *et al.* Effect of lentinan on cryopreservation of Hu sheep semen[J]. Animal Husbandry & Veterinary Medicine, 2022, 54(8): 9 – 13. (in Chinese).
[55] LIU CR, LI LX. Effect of lentinan on antioxidant function of loach (*Misgurnus anguillicaudatus*) under ammonia nitrogen stress[J]. Chinese Journal of Ecology, 2021, 40(11): 3701 – 3710. (in Chinese).
[56] ZI Y, ZHANG B, JIANG B, *et al.* Antioxidant action and protective and reparative effects of lentinan on oxidative damage in HaCaT cells[J]. Journal of Cosmetic Dermatology, 2018, 17(6): 1108 – 1114.
[57] LIU H, WANG Y, ZHANG W, *et al.* Lentinan extends lifespan and increases oxidative stress resistance through DAF-16 and SKN-1 pathways in *Caenorhabditis elegans*[J]. International Journal of Biological Macromolecules, 2022(202): 286 – 295.
[58] ZI Y, JIANG B, HE C, *et al.* Lentinan inhibits oxidative stress and inflammatory cytokine production induced by benzo(a)pyrene in human keratinocytes[J]. Journal of Cosmetic Dermatology, 2020, 19(2): 502 – 507.
[59] XU X, YANG J, NING Z, *et al.* *Lentinula edodes*-derived polysaccharide rejuvenates mice in terms of immune responses and gut microbiota [J]. Food & Function, 2015, 6(8): 2653 – 2663.
[60] ZHAO ZG, ZHANG LL, NIU CY, *et al.* Exogenous normal lymph reduces liver injury induced by lipopolysaccharides in rats[J]. Brazilian Journal of Medical and Biological Research, 2014, 47(2): 128 – 134.
[61] YAN J, LI S, LI S. The role of the liver in sepsis[J]. International Reviews of Immunology, 2014, 33(6): 498 – 510.
[62] DALVI YB, VARGHESE R, LAMROOD PY, *et al.* Mitigating acetaminophen-induced hepatotoxicity by using a water-alcohol extract of *Phellinus caryophylli* (Agaricomycetes) in a murine model[J]. International Journal of Medicinal Mushrooms, 2019, 21(4): 367 – 380.
[63] DU T, FANG Q, ZHANG Z, *et al.* Lentinan protects against nonalcoholic fatty liver disease by reducing oxidative stress and apoptosis via the PPARalpha pathway[J]. Metabolites, 2022, 12(1): 55 – 60.
[64] YANG Y, SONG S, NIE Y, *et al.* Lentinan alleviates arsenic-induced hepatotoxicity in mice via downregulation of OX40/IL-17A and activation of Nrf2 signaling[J]. BMC Pharmacology & Toxicology, 2022, 23(1): 16 – 26.
[65] SONG X, CAI W, REN Z, *et al.* Antioxidant and hepatoprotective effects of acidic-hydrolysis residue polysaccharides from shiitake culinary-medicinal mushroom *Lentinula edodes* (Agaricomycetes) in mice[J]. International Journal of Medicinal Mushrooms, 2021, 23(2): 85 – 96.
[66] SYN NL, TENG MWL, MOK TSK, *et al.* De-novo and acquired resistance to immune checkpoint targeting[J]. The Lancet Oncology, 2017, 18(12): e731 – e741.
[67] BAKIR HY, TOMIYAMA-MIYAJI C, WATANABE H, *et al.* Reasons why DBA/2 mice are resistant to malarial infection: expansion of $CD3^{int}$ $B220^{+}$ gammadelta T cells with double-negative $CD4^{-}$ $CD8^{-}$ phenotype in the liver[J]. Immunology, 2006, 117(1): 127 – 135.
[68] ISHIMOTO Y, TOMIYAMA-MIYAJI C, WATANABE H, *et al.* Age-dependent variation in the proportion and number of intestinal lymphocyte subsets, especially natural killer T cells, double-positive $CD4^{+}$ $CD8^{+}$ cells and $B220^{+}$ T cells, in mice[J]. Immunology, 2004, 113(3): 371 – 377.
[69] LI C, BAI X, WANG S, *et al.* Immunopotentiation of NKT cells by low-protein diet and the suppressive effect on tumor metastasis[J]. Cellular Immunology, 2004, 231(1 – 2): 96 – 102.
[70] XING J, LIU Z, HUANG Y, *et al.* Lentinan-modified carbon nanotubes as an antigen delivery system modulate immune response *in vitro* and *in vivo*[J]. ACS Applied Materials & Interfaces, 2016, 8(30): 19276 – 19283.
[71] JIANG Y, LI X, WU Y, *et al.* Effect of lentinan on Peyer's patch structure and function in an immunosuppressed mouse model[J]. International Journal of Biological Macromolecules, 2019(137): 169 – 176.
[72] WANG T, HE H, LIU X, *et al.* Mycelial polysaccharides of *Lentinula edodes* (shiitake mushroom) in submerged culture exert immunoenhancing effect on macrophage cells via MAPK pathway[J]. International Journal of Biological Macromolecules, 2019(130): 745 – 754.
[73] JEFF IB, FAN E, TIAN M, *et al.* *In vivo* anticancer and immunomodulating activities of mannogalactoglucan-type polysaccharides from *Lentinula edodes* (Berkeley) Singer[J]. Central European Journal of Immunology, 2016, 41(1): 47 – 53.
[74] XU WQ, WU YH, YU FL, *et al.* Inhibition mechanism of lentinan on the proliferation of breast cancer 4T1 cells xenograft model in mice[J]. Chinese Journal of Cancer Prevention and Treatment, 2021, 28(2): 111 – 116. (in Chinese).
[75] ZOU S, DUAN B, XU X. Inhibition of tumor growth by beta-glucans through promoting $CD4^{+}$ T cell immunomodulation and neutrophil-killing in mice[J]. Carbohydrate Polymers, 2019(213): 370 – 381.
[76] XU X, YANG J, LUO Z, *et al.* *Lentinula edodes*-derived polysaccharide enhances systemic and mucosal immunity by spatial modulation of intestinal gene expression in mice[J]. Food & Function, 2015, 6(6): 2068 – 2080.
[77] ZHU Z, YAN GM, HUANG YZ. Lentinan enhanced the cytotoxicity of epirubicin against bladder cancer cells[J]. Journal of Sun Yat-sen University (Medical Sciences), 2015, 36(4): 538 – 544, 598. (in Chinese).
[78] LU WQ, LIANG Y, ZHAI SW, *et al.* Preliminary study on effect of adding lentinan in diet of European eel (*Anguilla anguilla*)[J]. Feed Research, 2021, 44(21): 71 – 74. (in Chinese).
[79] ZHANG Z, WANG H, WANG K, *et al.* Use of lentinan to control sharp eyespot of wheat, and the mechanism involved[J]. Journal of Agricultural and Food Chemistry, 2017, 65(50): 10891 – 10898.
[80] TAMANG B, LIANG Q, BALAKRISHNAN B, *et al.* Astragalus shiitake: A novel functional food with high polysaccharide content and antiproliferative activity in a colorectal carcinoma cell line[J]. Nutrients, 2022, 14(11): 2333 – 2346.
[81] QI H, ZHANG WX, XIAO CL. Effect of *Strepiococcns* combined with lentinan on respiratory flora of mice with lung cancer after $PM_{2.5}$ exposure [J]. Chinese Journal of Microecology, 2022, 34(6): 638 – 643. (in Chinese).
[82] XIONG SM, REN ZP, SHI MJ, *et al.* Mechanism of lentinan on improving lung injury in septic mice based on mTOR pathway and autophagy [J]. Chinese Traditional and Herbal Drugs, 2022, 53(7): 2071 – 2077. (in Chinese).
[83] CHEN Q, PENG H, DONG L, *et al.* Activation of the NRF2-ARE signalling pathway by the *Lentinula edodes* polysaccharose LNT alleviates ROS-mediated cisplatin nephrotoxicity[J]. International Immunopharmacology, 2016(36): 1 – 8.
[84] ZHANG J, YANG CW, WANG Y, *et al.* Protective effect of lentinan on acute lung injury in sepsis mice[J]. Chinese Journal of Nosocomiology, 2018, 28(10): 1463 – 1468. (in Chinese).
[85] CHEN Q, LI JX. Protective effects of lentinan on bone metabolism in vitamin D deficient rats via vitamin D-fibroblast growth factor-23-klotho axis[J]. Modern Food Science and Technology, 2020, 36(1): 52 – 57. (in Chinese).
[86] HE J, LIU Z, JIANG W, *et al.* Immune-adjuvant activity of lentinan-modified calcium carbonate microparticles on a H5N1 vaccine[J]. International Journal of Biological Macromolecules, 2020(163): 1384 – 1392.
[87] ZHAO YM, YANG JM, LIU YH, *et al.* Ultrasound assisted extraction of polysaccharides from *Lentinula edodes* and its anti-hepatitis B activity *in vitro*[J]. International Journal of Biological Macromolecules, 2018 (107): 2217 – 2223.
[88] MA Q, PU Y, YUAN WQ, *et al.* Antidepressant effect of lentinan in chronically stressed mice and its possible mechanism[J]. Chinese Journal of Immunology, 2015, 31(3): 329 – 333. (in Chinese).
[89] LIU Q, DONG L, LI H, *et al.* Lentinan mitigates therarubicin-induced myelosuppression by activating bone marrow-derived macrophages in an MAPK/NF-kappaB-dependent manner[J]. Oncology Reports, 2016, 36 (1): 315 – 323.

[90] REN G, XU L, LU T, *et al.* Structural characterization and antiviral activity of lentinan from *Lentinula edodes* mycelia against infectious hematopoietic necrosis virus[J]. International Journal of Biological Macromolecules, 2018(115): 1202 – 1210.
[91] CHEN YY, GUAN YT, WANG X, *et al.* Protective effects of lentinan pretreatment on central nervous system injury in mice infected with Toxoplasma gondii[J]. Journal of Pathogen Biology, 2022, 17(2): 170 – 173. (in Chinese).
[92] LIU C, BAI YL, HE DQ. Effect of lentinan on postoperative inflammatory response and cognitive function in elderly mice with partial hepatectomy[J]. Chinese Traditional Patent Medicine, 2015, 37(9): 2032 – 2034. (in Chinese).
[93] PAN W, JIANG P, ZHAO J, *et al.* Beta-Glucan from *Lentinula edodes* prevents cognitive impairments in high-fat diet-induced obese mice: involvement of colon-brain axis[J]. Journal of Translational Medicine, 2021, 19(1): 54 – 70.
[94] ZHANG D, JIA Y, SUN X, *et al.* The Dectin-1 receptor signaling pathway mediates the remyelination effect of lentinan through suppression of neuroinflammation and conversion of microglia[J]. Journal of Immunology Research, 2022(2022): 3002304.
[95] SASAKI T, TAKASUKA N. Further study of the structure of lentinan, an anti-tumor polysaccharide from *Lentinula edodes*[J]. Carbohydrate Research, 1976, 47(1): 99 – 104.
[96] WANG KP, ZHANG QL, LIU Y, *et al.* Structure and inducing tumor cell apoptosis activity of polysaccharides isolated from *Lentinula edodes*[J]. Journal of Agricultural and Food Chemistry, 2013, 61(41): 9849 – 9858.
[97] QIAN Y, WANG D, FAN M, *et al.* Effects of intrinsic metal ions of lentinan with different molecular weights from *Lentinula edodes* on the antioxidant capacity and activity against proliferation of cancer cells[J]. International Journal of Biological Macromolecules, 2018(120): 73 – 81.
[98] KALETA B, GORSKI A, ZAGOZDZON R, *et al.* Selenium-containing polysaccharides from *Lentinula edodes*-Biological activity[J]. Carbohydrate Polymers, 2019(223): 115078.
[99] KLIMASZEWSKA M, GÓRSKA S, DAWIDOWSKI M, *et al.* Selective cytotoxic activity of Se-Methyl-Seleno-L-Cysteine- and Se-polysaccharide-containing extracts from shiitake medicinal mushroom, *Lentinula edodes* (Agaricomycetes)[J]. International Journal of Medicinal Mushrooms, 2017, 19(8): 709 – 716.
[100] WANG Y, HONG Q, CHEN Y, *et al.* Surface properties of polyurethanes modified by bioactive polysaccharide-based polyelectrolyte multilayers[J]. Colloids and Surfaces B: Biointerfaces, 2012(100): 77 – 83.
[101] WANG J, WANG HY, XIA XM, *et al.* Inhibitory effect of sulfated lentinan and lentinan against tobacco mosaic virus (TMV) in tobacco seedlings[J]. International Journal of Biological Macromolecules, 2013(61): 264 – 269.
[102] WANG L, WANG C, GAO X, *et al.* Purification, characterization and anti-aging capacity of mycelia zinc polysaccharide by *Lentinula edodes* SD-08[J]. BMC Complementary Medicine and Therapies, 2015(15): 111.

(From page 27)

[4] GRAY ER, Baimer JMT. Managing corporate image and corporate reputation[J]. Long Range Planning, 1998, 31(5): 695 – 702.
[5] VEH A, G BEL M, VOGEL R. Corporate reputation in management research: A review of the literature and assessment of the concept[J]. Business research, 2019, 12(2): 315 – 353.
[6] BARNETT ML, JERMIER JM, LAFFERTY BA. Corporate reputation: The definitional landscape[J]. Corporate Reputation Review, 2006, 9(1): 26 – 38.
[7] DEEPHOUSE DL. Media reputation as a strategic resource: An integration of mass communication and resource-based theories[J]. Journal of Management, 2000, 26(6): 1091 – 1112.
[8] ROBERTS PW, DOWLING GR. Corporate reputation and sustained superior financial performance[J]. Strategic Management Journal, 2002, 23(12): 1077 – 1093.
[9] CHEN JW, CHEN XL, SUN YM, *et al.* Research on the service marketing strategy of Haidilao restaurant[J]. Marketing Circles, 2020(3): 27 – 28.
[10] BARTON L. Crisis in organizations II, (2th ed.)[M]. Cincinnati, OH, 2001.
[11] COOMBS WT. Protecting organization reputations during a crisis: The development and application of situational crisis communication theory[J]. Corporate reputation review, 2007, 10(3): 163 – 176.
[12] FOMBRUN CJ. Reputation: realizing value from the corporate image[D]. Boston: Harvard Business School, 1996.
[13] FORMBRUN C, SHANLEY M. What's in a name? Reputation building and corporate strategy[J]. Academy of Management Journal, 1990, 33(2): 233 – 258.
[14] SHRUM W, WUTHNOW R. Reputational status of organizations in technical systems[J]. American Journal of Sociology, 1988, 93(4): 882 – 912.
[15] ROBERTS PW, DOWLING GR. Corporate reputation and sustained superior financial performance[J]. Strategic Management Journal, 2002, 23(12): 1077 – 1093.
[16] BLODGETT JG, HILL DJ, TAX SS. The effects of distributive, procedural, andinteractional justice on post-complaint behavior[J]. Journal of Retailing, 1997, 73(2): 185 – 210.
[17] COOMBS WT. Choosing the right words: The development of guidelines for the selection of the "appropriate" crisis-response strategies[J]. Management Communication Quarterly, 1995, 8(4): 447 – 476.
[18] COOMBS WT. The protection powers of crisis response strategies: Managing reputational assets during a crisis[J]. Journal of Promotion Management, 2006, 12(3/4): 241 – 260.
[19] GRONROOS C. Service quality the six criteria of good perceived service quality[J]. Review of Business, 1988, 9(3): 10 – 13.
[20] KELLEY SW, DAVIS MA. Antecedents to customer expectations for service recovery[J]. Journal of the Academy of Marketing Science, 1994, 22(1): 52 – 61.
[21] ZAID S, PALILATI A, MADJID R, *et al.* Impact of service recovery, customer satisfaction, and corporate image on customer loyalty[J]. The Journal of Asian Finance, Economics and Business, 2021, 8(1): 961 – 970.
[22] COOMBS WT, HOLLADAY SJ. An extended examination of the crisis situations: A fusion of the relational management and symbolic approaches[J]. Journal of Public Relations Research, 2001, 13(4): 321 – 340.
[23] DOWLING GR. Corporate reputation: Strategies for developing the corporate brand[M]. London, UK., 1994.
[24] BEJOU D, PALMER A. Service failure and loyalty: An exploratory empirical study of airline customers[J]. Journal of Services Marketing, 1998, 12(1): 7 – 22.
[25] ORSINGHER C, VALENTTNI S, DEANGELIS M. A meta-analysis of satisfaction with complaint handling in services[J]. Journal of the Academy of Marketing Science, 2010, 38(2): 169 – 186.
[26] HARRIS KE, GREWAL D, MOHR LA, BERNHARDT KL. Consumer responses to service recovery strategies: The moderating role of online versus offline environment[J]. Journal of Business Research, 2006, 59(4): 425 – 431.
[27] MAXHAM JG. Service recovery's influence on consumer satisfaction, positive word-of-mouth, and purchase intentions[J]. Journal of Business Research, 2001, 54(1): 11 – 24.